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Estimation of viable *Escherichia coli* O157 in surface waters using enrichment in conjunction with immunological detection

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Abstract

The use of a minimal lactose enrichment broth (MLB) in conjunction with immunomagnetic electrochemiluminescence detection (IM-ECL) was evaluated for the estimation of viable *Escherichia coli* O157 populations in surface water samples. In principle, *E. coli* O157 populations ($C_{\text{initial } E.\ coli\ \text{O157}}$) can be derived from enrichment data according to the equation: $C_{\text{initial } E.\ coli\ \text{O157}} = C_{\text{initial coliforms}} \times (C_{\text{final } E.\ coli\ \text{O157}}/C_{\text{final coliforms}})$, assuming that the growth rates and lag times of water-borne *E. coli* O157 and collective coliforms are sufficiently comparable, or at least consistent. We have previously described a protocol for determining $C_{\text{final } E.\ coli\ \text{O157}}$ in MLB-enriched water samples. In the present study, 80% of coliforms (red/pink colonies on MacConkey Agar) grew in MLB, indicating that this provides reasonably accurate estimates of $C_{\text{initial coliforms}}$. Estimates of $C_{\text{final coliforms}}$ were determined from turbidity data. Initial *E. coli* O157 populations ($C_{\text{initial } E.\ coli\ \text{O157}}$) were calculated for 33 Baltimore watershed samples giving a positive IM-ECL response. The majority of samples contained *E. coli* O157 concentrations of <1 cell per 100 ml. These data indicate that *E. coli* O157 are present in surface water samples but at very low levels. Growth rates for MLB-enriched coliforms were highly variable ($k = 0.47 \pm 0.13\ \text{h}^{-1}$, $n = 72$). There was no correlation between growth rates and any measured water parameter, suggesting that coliform populations in water samples are spatially and temporally unique. Although variability in growth rates was expected to yield some low values, the fact that most *E. coli* O157 concentrations were <1 suggests that other factor(s) were also responsible. Studies with *E. coli* O157:H7 and wild-type *E. coli* suggest that increased lag times due to starvation were at least partially responsible for the observed data. Based on estimates of $C_{\text{initial coliforms}}$ and $k_{\text{coliforms}}$, MLB was evaluated for sensitivity and quantitativeness. Simulated populations of *E. coli* O157:H7 at stationary phase varied from ca. 10^3 to 10^8 cells ml^{-1} enrichment culture. Although not suitable for quantitation, MLB enrichment in conjunction with IM-ECL can detect as few as one viable water-borne *E. coli*

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O157 cell per 100 ml surface water. Experiments are in progress to evaluate alternative media for sensitivity and quantitative detection of enterohemorrhagic *E. coli*.

Keywords: *E. coli* O157; Enterohemorrhagic *E. coli*; Water-borne pathogens; Pathogen detection; Immunological detection; Microbial water quality

1. Introduction

Enterohemorrhagic *Escherichia coli* (e.g., *E. coli* O157:H7) has emerged as a serious gastrointestinal pathogen (Mead et al., 1999). Although the predominant mode of transmission to humans appears to be contaminated meat or meat products, infection from contaminated water has also been documented. For example, one of the largest water-borne outbreaks of *E. coli* O157:H7 recently occurred in Walkerton, Ontario, Canada; out of 1346 reported cases of gastroenteritis, 167 were confirmed to be *E. coli* O157:H7 infections (Anonymous, 2000). Outbreaks associated with contaminated drinking water have also been reported in the U.S. (Olsen et al., 2002), Japan (Akashi et al., 1994) and Europe (Paunio et al., 1999).

Standard protocols for detection of enterohemorrhagic *E. coli* (EHEC) rely on enrichment and plating on selective media followed by biochemical and serological characterization (Dey and Lattuada, 1998). Such protocols, however, are not suitable for routine water quality analysis where speed, sensitivity and quantification are critical. Sensitivity and quantification are necessary to accurately evaluate public health risks, while speed is necessary to ensure that contaminated recreational or drinking waters are identified in a timely manner in order to minimize human exposure. Although the infectious dose for EHEC has not been definitively established (Kothary and Babu, 2001), epidemiological data suggest that relatively few cells are required for infection (Meng et al., 2001). Consequently, the ideal method should allow for quantitative detection of one viable EHEC per 100 ml of raw water, while analysis time should be consistent with current methods for generic *E. coli* (24 h).

Recently, a variety of molecular methods (immunological and PCR) have been developed which are potentially suitable for detection of water-borne EHEC. PCR-based methods detect one or more genes required for virulence (e.g., shiga-like toxin genes, attachment genes) while immunological-based methods typically utilize antibodies specific for the O157 serogroup. None of the methods, however, are capable of detecting one cell per 100 ml of raw water without either concentration or enrichment prior to analysis.

There are advantages and disadvantages to both strategies. Assuming relatively constant concentration/extraction efficiencies, concentration methods allow for quantitative detection. However, to achieve the desired sensitivity, an ca. 1000-fold concentration is necessary. This requires either a large initial volume of water or a small final volume. Depending on the raw water turbidity, this may result in substantial amounts of sediment, detritus or assorted microorganisms also being concentrated. To the extent that these interfere with analysis, additional sample preparation may be required (e.g., IMS). In addition, concentration methods result in detection of both viable and non-viable organisms, which may result in exaggerated risk assessments. Finally, concentration methods are not amenable to simultaneous processing of multiple water samples with limited time, money and labor resources.

An alternative strategy is filtration followed by enrichment (of the filter) for some population of water-borne bacteria (e.g., coliforms), including EHEC and/or *E. coli* O157. The advantages of enrichment are that (i) cell numbers can be magnified several orders of magnitude to maximize sensitivity, (ii) contaminants that pass through or adhere to the filter will not interfere with analysis and (iii) only viable organisms are detected. In addition, filtration/enrichment procedures are relatively inexpensive and multiple water samples can be processed simultaneously. The primary drawback of current enrichment procedures is that they provide only qualitative information (i.e., presence/absence).

In principle, *E. coli* O157 populations in raw water samples ($C_{\text{initial } E.\ coli\ \text{O157}}$) can be derived from enrichment data according to the equation:

$$C_{\text{initial } E.\ coli\ \text{O157}} = C_{\text{initial coliforms}} \times \left(C_{\text{final } E.\ coli\ \text{O157}} / C_{\text{final coliforms}}\right)$$

where $C_{\text{initial coliforms}}$ is the initial concentration of coliform bacteria in raw water samples which grow in the enrichment broth, $C_{\text{final } E.\ coli\ \text{O157}}$ is the concentration of *E. coli* O157 in the enrichment broth at stationary phase and $C_{\text{final coliforms}}$ is the concentration of coliform bacteria in the enrichment broth at stationary phase. This relationship assumes that the enrichment medium allows for growth of only the target population (coliform bacteria), and that the growth rates and lag times of water-borne *E. coli* O157 and collective coliforms are sufficiently comparable, or at least consistent. The magnitude of the error associated with estimating $C_{\text{initial } E.\ coli\ \text{O157}}$ will be dependent on the variability in growth rates and lag times for *E. coli* O157 ($k_{E.\ coli\ \text{O157}}$) vs. the collective coliform strains in water ($k_{\text{coliforms}}$), as well as the time required to reach stationary phase which is dependent on $C_{\text{initial coliforms}}$.

We have previously described a protocol for quantitative detection of *E. coli* O157 in a minimal lactose enrichment broth (MLB) with lactose as sole carbon source using immunomagnetic electrochemiluminescence (IM-ECL) for detection (Shelton et al., 2003). The protocol allowed for quantitative detection of 10^3 *E. coli* O157 cells ml^{-1} of MLB, which contained 10^8–10^9 coliform cells ml^{-1} at stationary phase.

In this manuscript, we report on the use of MLB-enrichment, in conjunction with IM-ECL, for the estimation of *E. coli* O157 populations in raw water samples. The overall goal of this research was to develop generic procedures for evaluating enrichment media with respect to sensitivity and quantitativeness.

2. Materials and methods

2.1. Bacteria, growth conditions and reagents

MacConkey Agar was purchased from Spiral Biotech (Providence, RI). Membrane fecal coliform (mFC) medium was purchased from Difco Laboratories (Detroit, MI). MLB consisted of the basal salts medium of Hylemon and Phibbs (1972) (50 mM potassium phosphate, 15 mM ammonium and trace nutrients) modified by the addition of (per liter) 8.5 g NaCl, 1.5 g Bacto bile salts (Difco) and 1.8 g lactose (5 mM). Phosphate-buffered saline (PBS) consisted of 150 mM potassium phosphate buffer (pH 7.2) and 150 mM NaCl; PBS-2 consisted of PBS with 0.1% azide. Diluent consisted of PBS-2 amended with 4% (w/v) bovine serum albumin (BSA) and 1% (v/v) thesit (polyoxyethylene 9 lauryl ether).

E. coli O157:H7 strain SEA13B88, originally isolated from Odwalla unpasteurized apple juice, was obtained from Dr. Pina Fratamico (USDA/ARS/ERRL, Wyndmoor, PA). *E. coli* strain ATCC 11775 was purchased from the American Type Culture Collection (Rockville, MD). ATCC 11775 is the control strain used for API products (Hazelwood, MO).

Streptavidin beads (2.8 μm; Dynabeads M-280™) were manufactured by DYNAL (Oslo, Norway) and purchased from IGEN International (Gaithersburg, MD). Biotin_LC_sulfoNHS ester and ORIGEN TAG-NHS ester [ruthenium (ii) tris_bipyridyl (referred to hereafter as TAG)] were purchased from IGEN International. The monoclonal antibody to O157 LPS used for cell capture was obtained as a liquid suspension from BioDesign International (Kennebunk, ME). The affinity purified polyclonal antibody to *E. coli* O157:H7 used for cell labeling with TAG was obtained from Kirkegaard and Perry Labs (KPL; Gaithersburg, MD).

2.2. Water collection

For growth rate studies, water samples (500 ml) were obtained weekly from various subcatchments of the Gwynns Falls, a 17,150-ha watershed which traverses an urban–rural land-use gradient (6.7% agriculture, 18.9% forest and 74.3% urban) in Baltimore City and Baltimore County, MD. These sites are the focus of an NSF urban Long-Term Ecological Research Project. The Gwynns Falls travels through numerous neighborhoods and public parks before draining to Baltimore Harbor and ultimately into the Chesapeake Bay. Water samples were collected from

July through September 2002. Samples were transported to the laboratory on ice the day of collection, refrigerated overnight and processed the following morning.

For lag time studies, a 10-l water sample was collected from Little Cove Creek, located in the Conococheague-Opequon watershed in south_central PA. The creek drains a watershed containing a combination of forest and pasture (dairy). The creek flows into the Potomac River near Hancock, MD. The water sample was stored at 25 °C until there were no detectable coliform bacteria (≤1 coliform bacterium 300 ml^{-1}).

2.3. Enrichment and growth rate studies

One hundred milliliters of water were filtered through a 47-mm diameter, 0.45-μm pore size sterile cellulose filter (Osmonics, Minnetonka, MN). The filters were placed into 10 ml of MLB and incubated overnight at 37 °C without shaking to facilitate anaerobic growth conditions. Preliminary studies indicated that *Pseudomonas* spp. were capable of rapid growth in MLB under aerobic conditions. Cultures were incubated at 37 °C because a preliminary screening study indicated that only 4 of 50 EHEC strains were capable of growth in MLB at 44.5 °C (unpublished data). Enriched samples were stored at 4 °C until IM-ECL analysis.

For growth rate determinations, water samples were processed as previously described, except that after 6–8 h of incubation, 10 and 100 μl of culture was transferred to 10 ml of fresh MLB. These samples were incubated overnight and turbidity monitored hourly the following day until stationary phase was achieved (Klett colorimeter with #66 filter). Growth rate constants (k) were derived from plotting the natural log of turbidity vs. time, assuming exponential growth ($C = C_o e^{kt}$).

Total coliform bacteria were determined by plating 100 μl of water samples onto MacConkey agar plates (two replicate plates) with an Autoplate 4000 spiral plater (Spiral Biotech). Plates were incubated overnight at 37 °C. Pink/red colonies were counted using an automated colony counter (Q-Counter; Spiral Biotech). To determine the ability of coliform bacteria to grow in MLB, 100 colonies from 9 different water samples were transferred to 10 ml of MLB. Colonies were designated as coliform or non-coliform based on a visual assessment of their color. Colonies were incubated at 37 °C and turbidity measured at ca. 16 and 40 h.

For selected samples ($n = 8$), turbidity at stationary phase (values ranging from 20 to 140) was correlated with cell numbers using a hemocytometer and phase contrast microscopy (500 ×). Linear regression of turbidity vs. cell numbers gave a value of 1 Klett unit = 5×10^6 cells ($r^2 = 0.94$).

2.4. Starvation (lag time) studies

Overnight cultures of *E. coli* O157:H7 strain SEA13B88 and generic *E. coli* strain 11775, grown to stationary phase in MLB at 37 °C, were used to inoculate 10 l of Little Cove Creek water. Cultures were diluted in PBS (10^4 cells of SEA13B88 ml^{-1} and 10^8 cells of 11775 ml^{-1}) and 1 ml of PBS added to the water to give final concentrations of ca. 1 cell ml^{-1} SEA13B88 and ca. 10^4 ml^{-1} 11775. Water was incubated at 25 °C and populations of strains 11775 and SEA13B88 determined at days 0, 4, 7 and 11.

Populations of *E. coli* O157:H7 SEA13B88 were determined as previously described, except that 10 or 100 ml of water were added to dry ingredients of MLB (phosphate, ammonium, salt, bile salts and lactose) and mixed thoroughly. Trace metals (1% v/v) were added after dissolution. Total coliforms (i.e., *E. coli* 11775 + SEA13B88) in water samples were determined by filtering 1, 10 or 100 ml of water samples (three replicate samples), transferring filters to mFC media, incubating overnight at 37 °C and manually counting blue colonies (Greenberg et al., 1998).

2.5. IM-ECL protocol

The preparation of biotinylated-and TAG-antibodies has been previously described (Shelton and Karns, 2001). IM beads were prepared in-house by incubating 2 mg of streptavidin beads for 1 h with 10 μg of biotinylated monoclonal antibody in 10 ml of diluent. Beads were harvested using a MPC-1 magnetic particle collector (Dynal, Oslo, Norway), suspended in 1 ml of PBS-2 providing a 10-fold concentration and stored at 4 °C until use.

The protocol for quantitative detection of *E. coli* O157 in MLB-enriched samples has been previously described (Shelton et al., 2003). Briefly, 100 μl of enrichment culture was added to the assay buffer consisting of 20 μl IM-beads, 100 μl diluent and 0.9 ml PBS-2 containing azide (2 tubes). To quantify competitive binding, 100 μl of enrichment culture plus 100 μl of a 10^5 cells ml^{-1} *E. coli* O157:H7 (strain SEA13B88) suspension (i.e., 10^4 cells) were added to the assay buffer (2 tubes).

After shaking for 3 h, beads were recovered for 15 min using a MPC-S magnetic particle collector (Dynal), the supernatant discarded and beads resuspended in 0.5 ml PBS-2 and 0.1 ml diluent. To label cells, 50 μl of the TAG (ORI_TAG) antibody (1–2 μg ml^{-1}) was added and the mixture shaken for two additional hours. Final total volume was 650 μl.

2.6. ECL instrumentation

Samples were analyzed using the ORIGEN manufactured by IGEN International (Gaithersburg, MD). Briefly, 550 μl of samples (85% of total volume) were pumped through a flow cell where the bead_cell_TAG complexes were magnetically captured on a platinum electrode, the sample washed to remove contaminants and unused reagents, and a voltage applied to create an electron transfer reaction in the presence of tripropylamine resulting in the emission of multiple photons from the Ru-chelate component of TAG. Adjustable instrument parameters were: assay gain of 100, instrument background subtracted and signal averaged. Approximate analysis time per tube was 75 s.

3. Results

3.1. Determination of $C_{initial\ coliforms}$ and $C_{final\ coliforms}$

Experiments were conducted to assess the percentage of coliform bacteria (defined as red/pink colonies on MacConkey agar after overnight incubation at 37 °C) which grew in MLB. Coliform ($n=56$) and non-coliform colonies ($n=44$), selected from nine watershed samples, were transferred to MLB tubes and incubated at 37 °C. An attempt was made to include as many different morphologically distinct colonies as possible. After overnight incubation (ca. 18 h), 60% of coliform tubes were turbid (>20 Klett units; $>10^8$ bacteria ml^{-1}), while after ca. 40 h 80% were turbid. After 40 h of incubation, only one non-coliform grew in MLB.

Final coliform concentrations at stationary phase were calculated from turbidity data. Based on hemocytometer counts, there was a linear correlation between turbidity and cell numbers (1 Klett unit = 5×10^6 cells; $r^2=0.94$; data not shown).

3.2. Estimation of $C_{initial\ E.\ coli\ O157}$

The concentration of *E. coli* O157 in MLB-enriched cultures ($C_{\text{final } E.\ coli\ O157}$) from 72 Baltimore watershed samples was determined as previously described (Shelton et al., 2003). Thirty-three samples gave a positive signal for the presence of *E. coli* O157 (data not shown). Based on determinations of $C_{\text{final coliforms}}$ (from turbidity data) and $C_{\text{initial coliforms}}$ (from MacConkey agar plate counts), initial *E. coli* O157 populations in raw water samples were calculated according to the equation:

$$C_{\text{initial } E.\ coli\ O157} = C_{\text{initial coliforms}} \times \left(C_{\text{final } E.\ coli\ O157}/C_{\text{final coliforms}}\right).$$

With a few exceptions, samples had apparent *E. coli* O157 populations <1 cell per 100 ml raw water, suggesting that growth rates and/or lag time were not comparable (Fig. 1).

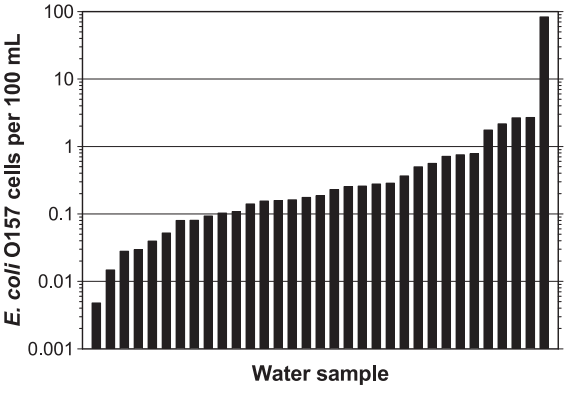

Fig. 1. Estimated *E. coli* O157 populations in selected Baltimore County watershed samples. Populations were calculated according to the equation: $C_{\text{initial } E.\ coli\ O157} = C_{\text{initial coliforms}} \times (C_{\text{final } E.\ coli\ O157}/C_{\text{final coliforms}})$.

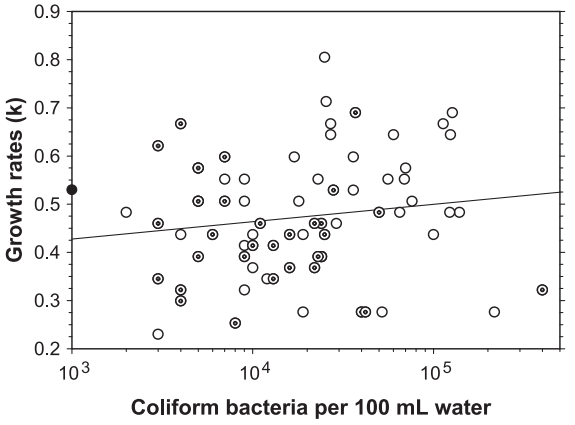

Fig. 2. Growth rates of water-borne coliform bacteria in Baltimore County watershed samples; symbols with internal circles represent samples in which *E. coli* O157 was detected. Growth rate for *E. coli* O157:H7 strain SEA13B88 is shown on the *y*-axis.

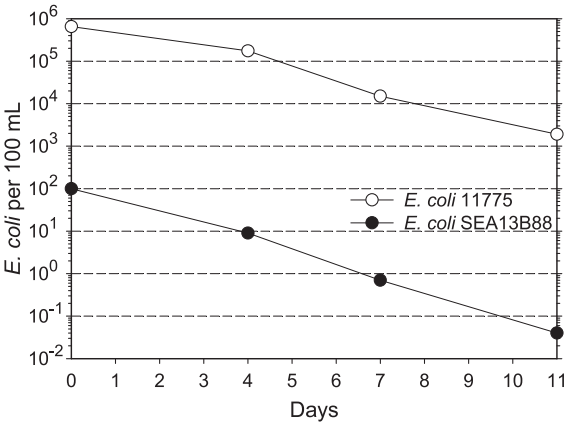

Fig. 3. Estimated *E. coli* O157:H7 strain SEA13B88 populations in Little Cove Creek water during starvation. *E. coli* O157:H7 populations were calculated according to the equation: $C_{\text{initial } E.\ coli\ O157} = C_{\text{wild-type } E.\ coli} \times (C_{\text{final } E.\ coli\ O157} / C_{\text{wild-type } E.\ coli})$.

3.3. Determination of $k_{E.\ coli\ O157}$ and $k_{coliforms}$

Growth rates were measured in MLB for 72 Baltimore watershed samples and for *E. coli* O157:H7 strain SEA13B88. Mean growth rates for MLB-enriched coliforms were 0.47 ± 0.13 h^{-1} (Fig. 2). Growth rates for water samples positive for the presence of *E. coli* O157 were comparable ($k = 0.43 \pm 0.11$ h^{-1}, $n = 33$). The growth rates were normally distributed (data not shown). No correlation was observed between growth rates and initial coliform concentrations (Fig. 2; $r^2 = 0.02$) or final coliform concentrations (data not shown). The growth rate for *E. coli* O157:H7 strain SEA13B88 was 0.53 h^{-1} (shown on *y*-axis of Fig. 2).

3.4. Effect of starvation on E. coli O157 estimations

Experiments were conducted to assess the affect of starvation on *E. coli* O157 recoveries from water samples using MLB enrichment. Ten liters of water were inoculated with ca. 1 cell of *E. coli* O157:H7 SEA13B88 ml^{-1} and ca. 10^4 *E. coli* 11775 ml^{-1}. Shortly after inoculation, the fraction of *E. coli* O157:H7 in MLB-enriched cultures, as determined by IM-ECL, was 0.016%. Based on a total coliform (i.e., *E. coli* 11775) count of 6500 cells ml^{-1}, the calculated initial *E. coli* O157:H7 population was 1 cell ml^{-1} (6500×0.00016), confirming that initial lag times and growth rates and for the two strains were similar. *E. coli* O157:H7 populations were estimated at 4, 7 and 11 days using the same protocol. Populations of both *E. coli* 11775 and SEA13B88 decreased exponentially (Fig. 3). Calculated *E. coli* O157:H7 populations at day 7 were <1 cell per 100 ml while at day 11 populations were <0.1 cell per 100 ml.

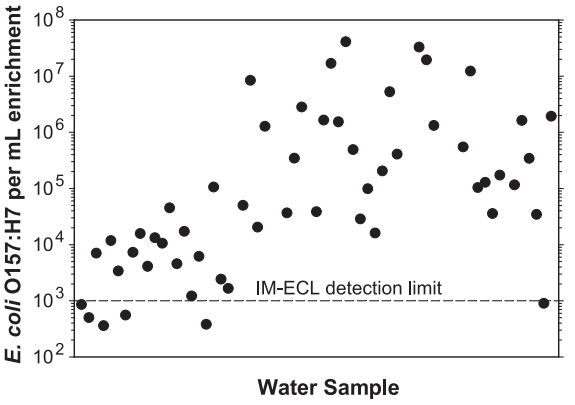

Fig. 4. Simulated stationary phase populations of *E. coli* O157:H7 SEA13B88 (cells ml^{-1}) based on growth rate and initial coliform data from Fig. 2 and assuming exponential growth.

3.5. Evaluation of sensitivity and quantitativeness

Based on determinations of $C_{\text{initial coliforms}}$ and $k_{\text{coliforms}}$, the sensitivity and quantitativeness of MLB was evaluated. The time required for each of the 72 Baltimore MLB-enriched water samples (Fig. 2) to reach stationary phase was calculated assuming exponential growth [$t = \ln(C_{\text{final coliforms}}/C_{\text{initial coliforms}})/k$]. Then, assuming one viable cell of *E. coli* O157:H7 strain SEA13B88 per 100 ml of raw water, the final concentrations of SEA13B88 at stationary phase ($C_{\text{final } E.\ coli\ \text{O157}}$) were calculated ($C_{\text{final } E.\ coli\ \text{O157}} = e^{0.53t}$; Fig. 4). Simulated populations of *E. coli* O157:H7 at stationary phase varied from ca. 10^3 to 10^8 cells ml^{-1} enrichment culture. The magnitude of the variability was a function of both differences in growth rates and initial coliform populations. Based on a detection limit of ca. 10^3 *E. coli* O157 ml^{-1} using IM-ECL, the majority of samples should give a positive response. However, as previously reported (Shelton et al., 2003), nonspecific binding of coliforms to IM beads can result in reduced capture of *E. coli* O157 cells, hence a higher detection limit.

4. Discussion

There is a pressing need for method(s) capable of measuring water-borne EHEC (e.g., *E. coli* O157:H7). In order to assess potential public health threats, method(s) need to be rapid, sensitive and quantitative. The ideal method should allow for quantitative detection of ≥1 viable EHEC per 100 ml of raw water, while analysis time should be consistent with current methods for generic *E. coli* (≤24 h). Various immunological and PCR-based methods have been developed for detection of EHEC generally and *E. coli* O157 specifically. However, none of the methods are capable of detecting one cell per 100 ml without either concentration or enrichment prior to analysis. Enrichment methods are typically used for qualitative analysis only; however enrichment media can, in principle, provide estimates of water-borne *E. coli* O157:H7 populations depending on variability in growth rates and lag times.

We have previously described a protocol for determining $C_{\text{final } E.\ coli\ \text{O157}}$ utilizing a MLB with lactose as sole carbon for enrichment and IM-ECL for detection (Shelton et al., 2003). The rationale for MLB was that the most selective medium would provide the greatest sensitivity. Previous data suggested that enrichment in MLB under anaerobic conditions was selective for lactose-fermenting coliform bacteria, including pathogenic *E. coli* (Shelton and Karns, 2001). The protocol allowed for a detection limit of $\geq 10^3$ *E. coli* O157 cells ml^{-1} of MLB containing 10^8–10^9 coliform cells ml^{-1} at stationary phase. An additional rationale for MLB was that the selective enrichment of lactose-fermenting (i.e., coliform) bacteria would allow for estimates of $C_{\text{initial coliforms}}$ to be obtained with standard coliform methods such as MacConkey or mFC media. LeJuene et al. (2001) reported good recoveries of water-borne *E. coli* O157 from typtic soya broth (TSB) enrichments incubated at 44.5 °C in conjunction with plating on sorbitol-MacConkey Agar supplemented with cefixime and tellurite (SMAC-CT). Although the fraction of *E. coli* O157 in the TSB enrichment was not reported, these data suggest that protein-based enrichment broths may also be effective.

We observed a reasonably good correlation between coliform counts on MacConkey Agar and growth in MLB ($C_{\text{initial coliforms}}$). Although no attempt was made to measure growth rates of individual coliforms, the time required for individual cultures to reach stationary phase (18–40 h) indicates that there was substantial variability in growth rates. Conversely, non-coliform bacteria did not grow in MLB. These results suggest that estimates of initial coliforms, as measured on MacConkey agar, probably exceed the numbers of coliforms which actually account for the majority of biomass at stationary phase; hence, a potential underestimate of initial *E. coli* O157 populations. However, this error is insignificant compared to the error from variability in growth rates or lag times.

Initial *E. coli* O157 populations ($C_{\text{initial } E.\ coli\ \text{O157}}$) were calculated for 33 Baltimore watershed samples [$C_{\text{initial } E.\ coli\ \text{O157}} = C_{\text{initial coliforms}} \times (C_{\text{final } E.\ coli\ \text{O157}}/C_{\text{final coliforms}})$]. With one exception, all samples contained ≤2 *E. coli* O157 cell per 100 ml. These data suggest that *E. coli* O157 are present in surface water samples but at very low levels; pure cultures of *E. coli* O157 isolated from these samples are currently being characterized to determine if they are *E. coli*

O157:H7 (EHEC). Although variability in growth rates would be expected to yield some values less than one, the majority of low values suggested that other factor(s) were responsible. Studies with *E. coli* O157:H7 and a wild-type *E. coli* suggest that increasing lag times due to starvation were at least partially responsible for the observed data. The fact that the calculated initial *E. coli* O157:H7 concentration, derived from MLB-enrichment data, accurately predicted the actual initial *E. coli* O157:H7 concentration (1 cell ml^{-1}) indicates that the growth rates and initial lag times for the *E. coli* O157:H7 and wild-type *E. coli* strains were similar. Therefore, since recoveries of <1 cell per enriched volume (100 ml) are theoretically impossible, the most plausible explanation for calculated *E. coli* O157:H7 concentrations <1 cell/100 ml is that lag times increased more rapidly for *E. coli* O157:H7 than the wild-type *E. coli*. Presumably, as critical enzymes are depleted during starvation, lag times increase in proportion to the time required for transcription and translation to regenerate critical metabolic pathways. Consequently, estimates of less than one viable *E. coli* O157 in surface waters after enrichment may be indicative of populations, which are in various stages of starvation. It should be feasible to measure actual lag times in different enrichment media as a function of starvation using immunological or PCR-based techniques so long as the initial population is detectable.

Growth rates for MLB-enriched water-borne coliforms were highly variable ($k = 0.47 \pm 0.13\ h^{-1}$). There was no correlation between growth rates and any measured water parameter. These data suggest that water samples are spatially and temporally unique, such that multiple samples will be required to adequately characterize a watershed with respect to pathogenic loading rates.

Based on estimates of $C_{\text{initial coliforms}}$ and $k_{\text{coliforms}}$, the sensitivity and quantitativeness of MLB was evaluated assuming a single cell of *E. coli* O157:H7 ($k = 0.53\ h^{-1}$) per 100 ml. There was substantial variability in simulated *E. coli* O157:H7 stationary phase concentrations (10^3–10^8 cells ml^{-1} enrichment culture). Although not suitable for quantification, MLB enrichment in conjunction with IM-ECL can detect as few as one viable water-borne *E. coli* O157:H7 cell per 100 ml surface water, based on a detection limit of 10^3 cells ml^{-1} MLB. Note that detection limits will vary depending on growth rates of specific *E. coli* O157:H7 strains and the efficiency of IM-ECL detection.

5. Conclusions

The method described here allows for evaluation of enrichment media for sensitivity and quantitativeness, assuming information on the concentration of cells, which grow in the enrichment medium and growth rates. We evaluated a minimal lactose broth (MLB) for enrichment in conjunction with immunological analysis (IM-ECL) for detection and enumeration of *E. coli* O157 in watershed samples. Although not suitable for quantification, the protocol allowed for detection of as few a one viable water-borne *E. coli* O157 cell per 100 ml surface water. Experiments are in progress to evaluate alternative media (e.g., MLB + yeast extract, EC broth, TSB) for sensitivity and quantitativeness.

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